THE EFFECT OF PROGESTERONE ON THE MOUSE OVARY AS INFLUENCED BY GESTATION [1]

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TWO PLATES (ELEVEN FIGURES)

Beard (1897) and Prenant (1898 a, 1898 b) were the first to put forward the theory that the corpus luteum of pregnancy is in some way responsible for the fact that no follicles mature and no corpora lutea are formed as a rule during the gestation period. This conception appeared to receive support from the experiments of Hill and Parkes ('32), who found that the corpora lutea of gestation tend to involute if a second set of corpora lutea is produced in pregnant rabbits by the administration of a gonadotrophic extract prepared from the urine of pregnant women. The authors concluded that the newly formed corpora "appear to exercise an adverse influence on the existing corpora lutea."

Numerous investigators have attempted to come closer to an understanding of the action of corpora lutea on the remaining ovarian tissue by studying the ovarian changes induced by the administration of corpus luteum implants or extracts. The findings of the early investigators were inconclusive because they used crude preparations which contained little if any actual corpus luteum hormone (Pearl and Surface, '14; Herrmann and Stein, '16; Corner and Hurni, '18; Haberlandt, '22; Cocchi, '32; Gostimirovic and Krämer, '32).

Even the observations of those investigators who used partially purified active corpus luteum preparations are

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contradictory. Thus Bates et al. ('35) claimed that corpus luteum hormone exerts an adverse influence on active mature fowl ovaries. Papanicolaou ('24, '26) noted that in the ovaries of mature guinea pigs, treatment with a lipid fraction of corpora lutea causes a delay in ovulation accompanied by the disappearance of corpora lutea and marked follicular atresia. Macht et al. ('29) found that treatment with a corpus luteum extract decreased the number of mature follicles and caused follicular atresia in the guinea pig. In the mouse, Patel ('30) noted that a corpus luteum hormone-containing extract may lead to continuous anestrus without any histological evidence of an ovarian disturbance. Several authors noted that in rabbits, corpus luteum extracts inhibit ovulation and corpus luteum formation following mating (Kennedy, '25; Mahnert, '30; Fels, '34; Makepeace et al., '36, '37). Knaus ('24) claimed that if mature female rats are treated with a corpus luteum extract, they remain sterile and histological studies of their ovaries indicate that the sterility is due to the fact that although maturation of the follicles is not interfered with, ovulation is prevented by this treatment. Hisaw et al. ('28) also reported the presence of large follicles and the absence of recent corpora lutea in the ovaries of rats treated with an active corpus luteum extract. Gley ('28 a, '28 b, '28 c), on the other hand, claimed that such treatment leads to disappearance of mature follicles though corpora lutea remain plentiful, while Abe ('31, '32) stated that both corpora lutea and follicles disappear under the influence of corpus luteum hormone treatment in the rat.

The observations of recent authors who used crystalline progesterone are likewise somewhat contradictory. Selye et al. ('36) administered 4 mg. of synthetic progesterone daily to normally cyclic rats. They noted immediate cessation of the vaginal cycles and autopsy on the thirteenth day of treatment revealed atrophic ovaries. They concluded that progesterone inhibits the development of the ovaries. On the other hand, McKeown and Zuckerman ('37) claimed to have obtained exactly opposite results inasmuch as they believed they had

seen corpus luteum formation as a result of progesterone treatment, which would indicate that this hormone actually stimulates the ovary. It is noteworthy, however, that in this experimental series, only 1 mg. of progesterone was admistered daily, that is to say, one-fourth of the dose used by Selye et al. ('36). Since our own unpublished data indicate that 1 mg. per day is not sufficient to inhibit ovulation completely in the adult rat, it seems possible that the recent corpora lutea observed by McKeown and Zuckerman in their progesterone treated animals were not formed as a result of the treatment but in spite of it. In fact, since these investigators used post-pubertal rats, we see no reason to assume that the recent corpora lutea observed in the ovaries at autopsy should have been produced by the progesterone treatment. McKeown and Zuckerman argued that their theory, according to which progesterone causes ovulation and corpus luteum formation, receives further support through the observations of Shapiro ('36), Shapiro and Zwarenstein ('37) and Zwarenstein ('37), who found that treatment with this hormone elicits ovulation in the toad, Xenopus laevis. It must be borne in mind, however, that in the case of this amphibian, not only progesterone but a great many other sterols have been shown by the above-mentioned investigators to cause extrusion of ova even from the excised ovary kept in vitro in Ringer's solution. In this case, the mechanism of ovulation is obviously different from the pituitary-controlled ovarian response in the rat. Dempsey ('37) and Dempsey et al. ('36) claimed that a single dose of 0.2 international units of progesterone delays the occurrence of the next expected ovulation in mature normally cyclic guinea pigs and that daily administration of 0.05 international units prevents ovulation during the entire duration of the treatment. The ovaries of these animals contained no corpora lutea but many atretic and some normal mature follicles. The authors concluded that progesterone prevents ovulation and corpus luteum formation without interfering with follicular growth. Experiments on the rabbit led Peraus ('36) to conclude that the life span of corpora lutea of pseudo-pregnancy

is not significantly altered by progesterone and Spanio ('38) observed no change in the ovaries of rabbits following seven daily injections of 1 mg. of this substance. On the other hand, pretreatment with progesterone inhibits ovulation and the formation of corpora lutea following subsequent mating as shown by Makepeace et al. ('36, '37) thus confirming the above mentioned findings of earlier investigators who had used corpus luteum extracts.

Since we believed that the dosage has much to do with the discrepancies in the results reported in the literature, we decided to repeat these experiments in mice, using relatively large doses of progesterone. We chose the mouse as an experimental animal because Selye et al. ('33 a, '33 b) found that unlike in most other animals, the involution of the corpora lutea is particularly rapid in hypophysectomized mice, a fact which was confirmed by Leblond and Nelson ('37 a). It seemed likely therefore, that if the involution of the corpora following progesterone treatment is due to an inhibition of pituitary function, it should be particularly obvious in this species.

Another series of experiments to be reported here deals with the action of progesterone on the ovary of the pregnant mouse. This appeared to be of interest since it was found that both in the rat (Selye et al. '33 c, Pencharz and Long, '33), and in the mouse (Selye et al. '34; Leblond and Nelson, '37 a, '37 b) hypophysectomy does not interfere with the maintenance of the corpora lutea of gestation from which it was concluded that the latter are probably maintained by a placental hormone and are consequently independent of the hypophysis. The work of Astwood and Greep ('38) gave important additional evidence in favor of this conception, since these authors showed that the corpora lutea of non-pregnant hypophysectomized rats may be maintained in a functional condition by rat placenta extracts. Astwood and Greep ('39) suggested terming the gonadotrophic hormone of the rat placenta 'murine cyonin.' In view of these findings, it appeared of interest to establish whether the corpora lutea of

pregnancy are also resistant to progesterone. It was felt that if the corpora lutea of the cycle involute while those of gestation prove resistant to progesterone, this would furnish further evidence in favor of the conception that the stimuli responsible for the maintenance of these two types of corpora lutea are not identical.

EXPERIMENTAL

Our first experiment was concerned with the action of progesterone on the ovaries of normally cyclic mice. For this and all other experiments reported in this paper, black mice of the Bar Harbor strain designated as C57 were used. In the first series, six non-pregnant adult females received 1 mg. of progesterone [2] dissolved in 0.2 cc. of Mazola oil daily by way of subcutaneous injections for 5 days. They were sacrificed on the sixth day. Each of these animals thus received a total of 5 mg. of progesterone. Seven normal adult non-pregnant controls were killed at the same time and the ovaries of all these animals were weighed and histologically examined. The first two columns of our table summarize the ovarian weights and it will be seen that the average ovarian weight in the normal group was 9 mg. and in the progesterone-

TABLE 1

Table showing the effect of progesterone on ovarian weight in pregnant and non-pregnant mice. The ovarian weights are expressed in milligrams

NON-PREGNANT UNTREATED	NON-PREGNANT PROGESTERONE TREATED	PREGNANT UNTREATED	PREGNANT PROGESTERONE TREATED
9	3	12	13
9	3	13	9
8	4	17	12
9	6	12	10
9	5	17	12
10	3	11	15
7		9	9
		17	
		14	
Average 9	4	14	11

[2] The progesterone was supplied by the Schering Corporation of Bloomfield, N. J., through the courtesy of Drs. G. Stragnell and E. Schwenk.

treated group, 4 mg. The ovaries of the treated animals were different from the normals even in their macroscopical appearance inasmuch as they contained no visible corpora lutea and their color was brownish yellow instead of the normal grayish pink. Histological examinations showed that while normal corpora lutea and follicles were present in the ovaries of untreated mice, mature follicles and recent corpora lutea were invariably absent in the progesterone-treated group. In a few cases, vestiges of involuting corpora were still detectable but in most ovaries, the involution of the corpora lutea was complete. Figure 1 shows a normal control ovary with well-developed corpora lutea at low magnification. Figure 2 shows the ovary of a progesterone-treated animal containing medium sized atretic follicles and involuting corpora lutea. The interstitial tissue of the ovary likewise undergoes marked alterations under the influence of progesterone treatment. As shown in figure 3, the interstitial cells and 'theca nests' in normal mouse ovaries consist of cells having a fair amount of cytoplasm. On the other hand, in the progesterone-treated ovary represented in figure 4, the interstitial tissue is very atrophic and as a result of marked involution of the cytoplasm, cell nuclei with dense chromatin are predominant in the entire field. When examined under very high magnification, it was noted that between the atrophic interstitial cells, large light cells appear in some regions of the ovary. These have a vacuolated cytoplasm and contain a certain amount of a yellowish brown pigment which gives a positive Prussian blue reaction. It seems probable that the appearance of the pigment-containing cells is the cause of the yellowish discoloration of these ovaries which was observed macroscopically. Figures 5 and 6 are high magnifications of corpora lutea from the ovaries of untreated and progesterone-treated animals respectively. It will be observed that the corpus luteum of the treated animal shows obvious signs of involution although this was the best preserved corpus luteum in the entire treated group.

In a second experimental series, seven pregnant mice were treated with 1 mg. of progesterone daily for 8 days and killed on the ninth. In all cases, treatment was begun during the second half of gestation and three of the animals delivered normal litters on the eighth and three others on the ninth day of the experiment. The remaining animal was still pregnant when killed but judged from the appearance of the fetuses, must have been very close to delivery. Although the actual date of conception was not known in these cases, it is evident that since pregnancy in the mouse is of about 21 to 22 days duration, the treatment was administered during the second half of the gestation period. For control purposes, six pregnant non-treated animals were killed on the day of delivery and three others on the twentieth day of pregnancy. The third and fourth columns in our table indicate that the average weight of the ovaries was 14 mg. in the untreated and 11 mg. in the treated group. This would seem to indicate that progesterone caused at least a slight degree of ovarian atrophy even in this series, but considering the great individual variations, it is doubtful whether the difference is significant enough to reach such a conclusion. The gross weight of the ovary during pregnancy depends almost entirely on the number of corpora lutea and it was noted that most of the animals in the treated group had fewer corpora and fewer fetuses than those of the control group. Since treatment was begun during the second half of gestation, the number of ovulating follicles transformed into corpora lutea could not have been altered by the treatment and as the size and the histological appearance of the individual corpora was the same in both groups, it seems most unlikely that progesterone exerted no effect on the ovary of the pregnant mice. Figures 7 and 8 show low magnification views of ovaries of untreated and progesterone-treated mice respectively. It will be observed that the general size of the corpora lutea is approximately the same in both instances. Figures 9 and 10 show high magnifications of the corpora lutea from these ovaries. The structure of the corpora lutea and the appearance of their cells

are not noticeably altered by the progesterone treatment. The interstitial cells were likewise not influenced by progesterone in this series.

Concerning the maturation of follicles, our results were less clear cut. In the non-pregnant, untreated animals, large follicles were often observed, while in the treated non-pregnant group only small or medium-sized follicles were seen and there was considerable follicular atresia. In the two pregnant groups, large follicles were never observed and the amount of follicular atresia was approximately the same in the treated and the untreated animals.

It should be emphasized particularly that the administration of these large doses of progesterone did not interfere either with delivery itself or with the onset of lactation on the day of delivery. This is all the more noteworthy since numerous investigators regarded the uterine contractions which extrude the fetuses and the onset of milk secretion at the end of gestation as due to the involution of the corpora lutea and the consequent drop in the corpus luteum hormone content of the blood. Although our experiments do not give any indication concerning the factors responsible for the birth mechanism or the initiation of milk secretion, they seem to indicate that if such large doses of progesterone as were given in the present series are unable to prevent delivery and the onset of milk secretion at this time, one can hardly regard these processes as due to a sudden discontinuation of corpus luteum hormone production unless one assumes that a corpus luteum hormone other than progesterone is involved. Ehrhardt and Hardt ('37) found that a total dose of one-tenth to one-half rabbit unit of corpus luteum hormone administered in the shape of an extract, suffices to terminate pregnancy in the mouse. They concluded that great caution is indicated if this hormone is to be given to pregnant women. Our experiments show, however, that even as high a total dose as 8 mg. of pure progesterone does not interfere with gestation in this species, so that there is no reason for fear of abortion if this compound is used.

SUMMARY AND CONCLUSIONS

Experiments on the adult mouse indicate that daily administration of 1 mg. of progesterone for 5 days suffices to cause corpus luteum involution and marked ovarian atrophy. In pregnant mice, even eight daily injections of 1 mg. of progesterone do not cause significant ovarian atrophy or any detectable change in the corpora lutea of gestation. Since hypophysectomy likewise causes rapid involution of the corpora lutea in the non-pregnant mouse but remains without detectable influence on the corpora lutea of gestation, it seems possible that the ovarian atrophy caused by progesterone is due to an inhibition of the gonadotrophic function of the hypophysis. We are also led to conclude that the gonadotrophic hormone of the placenta which maintains the corpora lutea of gestation is not inhibited by progesterone.

Delivery and the onset of lactation are not prevented by massive doses of progesterone. This finding is not in accordance with the assumption that discontinuation of corpus luteum hormone production is the cause of delivery at term and of the initiation of milk secretion.

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PLATE 1

EXPLANATION OF FIGURES

1 Ovary of an untreated mouse showing normal corpora lutea of the cycle.

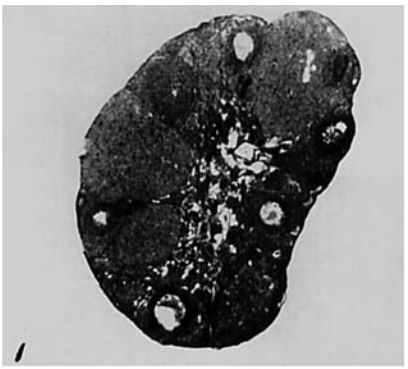
2 Ovary of progesterone treated non-pregnant mouse showing atrophic corpora lutea and atretic follicles.

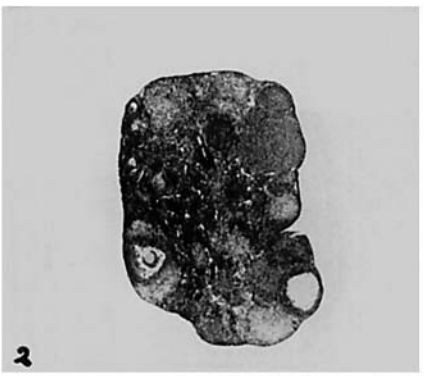
3 Interstitial tissue in the ovary of an untreated mouse showing light cells rich in cytoplasm.

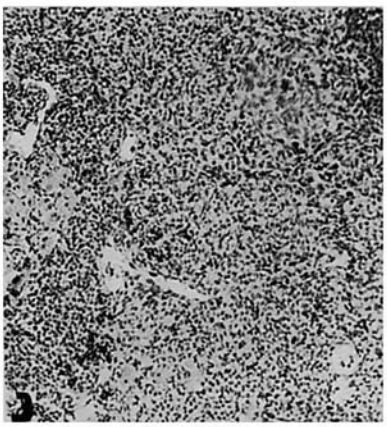
4 Interstitial tissue in the ovary of a progesterone treated non-pregnant mouse showing atrophy. Note the dense nuclei and the scarcity of cytoplasm.

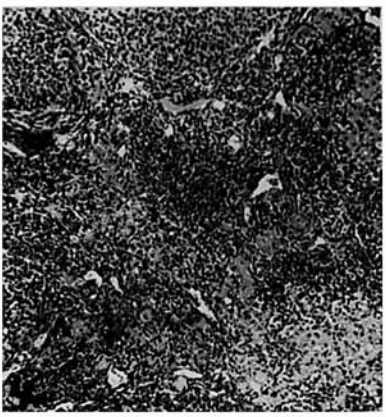
5 Normal corpus luteum of an untreated non-pregnant mouse.

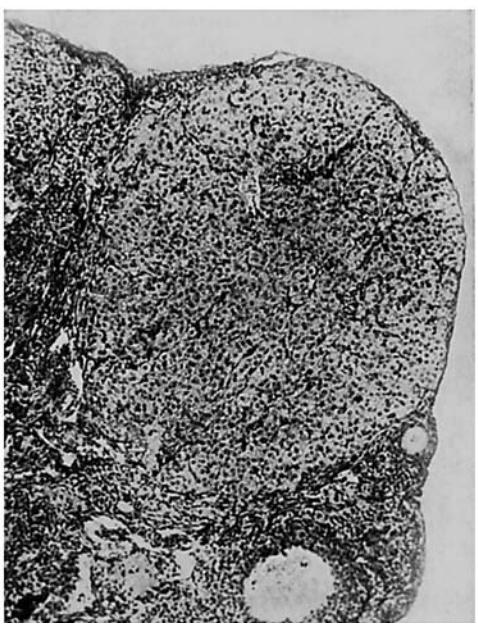
6 Involuting corpus luteum of a progesterone treated non-pregnant mouse.











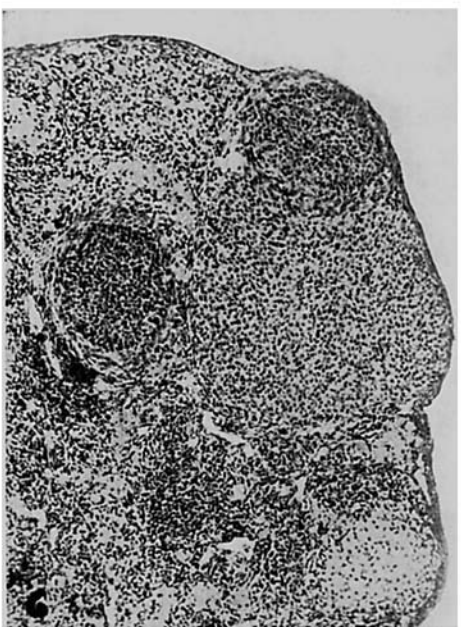


PLATE 2

EXPLANATION OF FIGURES

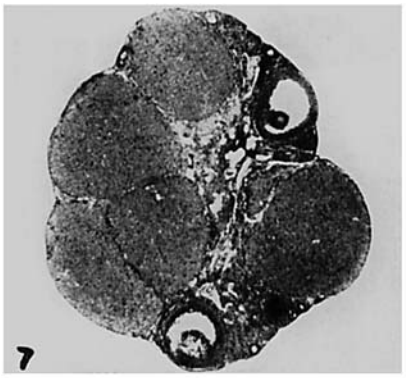
7 Ovary of an untreated pregnant mouse showing four corpora lutea of gestation.

8 Ovary of a progesterone treated pregnant mouse with two corpora lutea of approximately the same size as those seen in the ovary of the untreated pregnant control shown in figure 7.

9 Normal corpus luteum of gestation in the ovary of an untreated mouse.

10 Normal corpus luteum of gestation in the ovary of a progesterone treated mouse. Its structure is the same as that of the untreated pregnant control shown in figure 9.

11 Fully lactating mammary gland of a progesterone treated rat on the day of delivery. It will be noted that the galactophores and acini are full of milk in spite of the treatment.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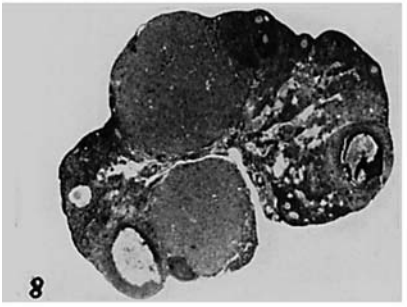
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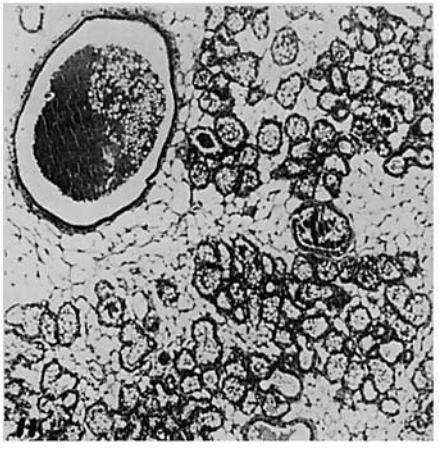
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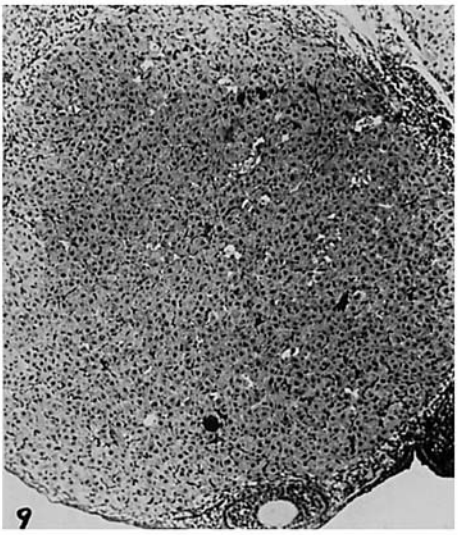
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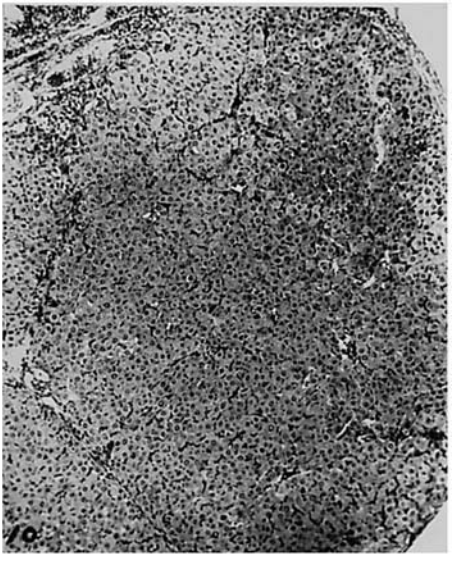
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